

LHRH_a Spawning Hormone Clinical Field Trials - INAD 8061

2009 Annual Summary Report on the Use of LHRH_a in Clinical Field Efficacy Trials

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Summary

Spawning aids such as luteinizing hormone-releasing hormone analogue (LHRH_a), human chorionic gonadotropin, and common carp pituitary are routinely used in aquaculture to induce gamete maturation in fish to enhance fish propagation programs. The U.S. Food and Drug Administration has authorized the use of LHRH_a under the Compassionate Investigational New Animal Drug (INAD) Exemption #8061 for the purpose of gathering efficacy data to support a new animal drug approval for LHRH_a. In calendar year 2009 (CY09), 50 trials were conducted under this INAD to evaluate the efficacy of LHRH_a to induce gamete maturation in a variety of fish species. Trials involved 2,314 treated fish and 317 control fish and were conducted at 25 different hatcheries, including five U.S. Fish and Wildlife Service fish hatcheries, one United States Department of Agriculture - Agriculture Research Station, 11 state hatcheries, seven private hatcheries, and one tribal hatchery during this period. Efficacy was determined by whether or not treated fish produced or yielded more eggs or milt than untreated fish. Overall results from trials conducted in CY09 showed that

treatments appeared efficacious in approximately 88% of the trials, ineffective in 10% of the trials, and was inconclusive in 2% of the trials.

Introduction

The use of hormones to induce spawning in fish is critical to the success of many federal, state, private, and tribal fisheries programs. A wide variety of programs, including many that involve the restoration of threatened/endangered species, are dependent upon hormone treatment to complete final gamete maturation and ensure successful spawning.

The time of spawning is by its own nature a stressful period for all fish species. Both sexes are undergoing significant changes in physiology, morphology, and behavior (Hoar, 1969). The additional handling of fish required during the spawning process complicates an already delicate situation. This is particularly true for wildstock species that must endure the added stresses of capture, handling, and confinement in an unnatural environment. In fact, with respect to some wildstock species, the stress of capture alone is often sufficient to cause complete reproductive failure unless spawning is induced by hormone treatment. Hormone treatment in a variety of fish species is essential to ensure optimal spawning success.

Studies have shown that final gamete maturation (ovulation and spermiation) in fish can be induced by the administration of a variety of hormones (Donaldson and

Hunter 1983; Goetz 1983). Recent investigations have found luteinizing hormone-releasing hormone analogue (LHRH_a) to be one of the most effective means of inducing final gamete maturation. This compound is a synthetic gonadotropin releasing hormone that is similar in structure to native luteinizing hormone-releasing hormones. LHRH_a is an attractive choice as it has both a high biological activity and low species specificity, making it appropriate for use on a variety of fish species (Coy et al. 1974). Although the use of LHRH_a as a tool to enhance broodstock spawning success is relatively new, it has already had a significant, positive impact on fisheries programs nationwide.

Purpose of Report

The purpose of this report is to summarize the results of LHRH_a field efficacy trials conducted under INAD exemption #8061 in CY09. Furthermore, it is expected that these data will be used to enhance the existing LHRH_a database that has been established from previous years trials for the purpose of developing an appropriate label claim for the use of this new drug.

Facilities, Materials, and Treatment Procedures

1. Facilities

Field efficacy trials were conducted at 25 different fish culture facilities during CY09, including five U.S. Fish and Wildlife Service fish hatcheries, one United States Department of Agriculture - Agriculture Research Station, 11 state

hatcheries, seven private hatcheries, and one tribal hatchery. Water temperature during treatments at the various testing facilities ranged from 42.0 to 82.0°F.

Overall mean treatment temperature from all trials was 62.7 °F.

2. Chemical material

Western Chemical Inc. of Ferndale, WA an Aquatic Life Sciences Company was the supplier for all LHRH_a used in trials conducted during the reporting period.

3. Drug dosages

The Study Protocol authorized the use of up to 100 ug LHRH_a/kg fish body weight (bw). During this reporting period, the drug doses used ranged from 5 to 100 ug LHRH_a/kg fish body. LHRHa was administered as either a single injection or as a series of two injections.

Fish Species and Sex Treated

1. Fish Species Treated

Field efficacy trials were conducted on 19 different fish species under INAD #8061 during the reporting period, including the following two salmonid and 17 non-salmonid species:

Salmonids

Gila trout *Oncorhynchus gilae*

rainbow trout *O. mykiss*

Non-salmonids

alligator gar *Lepisosteus spatula*

American shad *Alosa sapidissima*

Atlantic sturgeon *Acipenser oxyrhynchus*

lake sturgeon *A. fulvescens*

Siberian sturgeon *A. baerii*

starry sturgeon *A. stellatus*

white sturgeon *A. transmontanus*

blue catfish *Ictalurus furcatus*

channel catfish *I. punctatus*

flathead catfish *Pylodictis olivaris*

largemouth bass *Micropterus salmoides*

paddlefish *Polydon spathula*

pallid sturgeon *Scaphirhynchus albus*

shovelnose sturgeon *S. platyrhynchus*

sauger *Stizostedion canadense*

striped bass *Morone saxatilis*

walleye *Sander vitreus*

2. Gender of treated fish

LHRH_a was used on 2,066 female and 248 male fish during the reporting period. Typically, females were treated with spawning hormone to shorten the gamete maturation period (i.e. advance maturation), while males were treated to ensure that sufficient milt would be available for egg fertilization.

Data Collected

1. Primary response variable (Maturation)

The primary response variable for evaluating the effect of LHRH_a on fish was the percentage of ripe fish following treatment. These percentages reflected the number of female fish that ovulated and the number of male fish that reached active spermiation.

2. Egg development and milt evaluation

Secondary response variables for females included the relative number of eggs that reached the eyed stage and the number hatched. Secondary response variables for males included the volume of milt (ml) available from individual fish and an evaluation of milt motility (percent motile spermatozoa).

3. Spawning interval

The time period between the last treatment and when fish were spawned or evaluated for ripeness was also documented. In the case of females, which in some cases received a priming dose followed a short time later (12 - 24 hrs) by a resolving dose, the spawning interval was defined as the time period between administration of the resolving dose and spawning.

Discussion of Study Results

1. General observations on the efficacy of LHRH_a to induce gamete maturation in salmonid and non-salmonid fish (Note: Tables 1 - 2 provides summaries of all efficacy trials; Table 3 lists the number of treatment trials, number of fish and species treated, and treatment regimens used; and Table 4 describes all trials conducted during CY09 under INAD #8061.)

A. Efficacy of LHRH_a on male fish treated at a dosage between 5 and 100 ug/kg body weight (1 - 2 injections)

Treated male fish were used in 17 trials and injected one to two times with LHRH_a at a dosage between 5 and 100 ug/kg body weight (Table 1). Below is the treatment regimen used when treating fish species with this dosage range of LHRH_a:

1. Dose: 5 - 25 ug/kg

Gila trout, paddlefish, largemouth bass, lake sturgeon, pallid sturgeon, shovelnose sturgeon, and white sturgeon were used in nine trials and injected 1 - 2 times with LHRH_a; control fish were used in four trials.

Results showed there was a 0 - 100% spermiation in treated fish; and 0 - 85% spermiation in control fish. Overall, results indicated that treatment appeared effective in eight trials and was ineffective in one trial.

2. Dose: 26 - 50 ug/kg

Atlantic sturgeon, paddlefish, and shovelnose sturgeon were used in four trials and injected 1 time with LHRH_a; no control fish were used. Results showed there was a 31 - 100% spermiation in treated fish. Overall, results indicated that treatment appeared effective in all trials.

3. Dose: 100 ug/kg

American shad, alligator gar, flathead catfish, and paddlefish were used in four trials and fish were injected 1 - 2 times with LHRH_a; control fish were used in two trials. Results showed that there was a 0 - 100% spermiation in treated fish; and a 33 - 100% spermiation in control fish. Overall, treatment appeared to be effective in three trials and ineffective in one trial.

Overall treatment resulted in a 0 - 100% level of spermiation in the male treated fish, compared to a 0 - 100% level of spermiation in control fish. Treatment appeared effective in 15 trials and ineffective in two trials.

B. Efficacy of LHRH_a on female fish treated at a dosage between 5 and 100 ug/kg body weight (1 - 2 injections)

Treated female fish were used in 33 trials and injected 1 - 2 times with LHRH_a at a dosage between 5 and 100 ug/kg body weight (Table 2). Below is the treatment regimen used when treating fish with this dosage range of LHRH_a:

1. Dose: 5 - 25 ug/kg

Gila trout, rainbow trout, largemouth bass, paddlefish, shovelnose sturgeon, striped bass, and white sturgeon were used in nine trials, fish were injected 1 or 2 times with LHRH_a; four trials involved control fish. Treatment results showed that there was 0 - 100% ovulation in treated fish; and 0 - 95% ovulation in control fish. Overall, treatment appeared effective in eight trials and ineffective in one trial.

2. Dose: 26 - 50 ug/kg

Lake sturgeon, paddlefish, pallid sturgeon, shovelnose sturgeon, Siberian sturgeon, starry sturgeon, striped bass, sauger, walleye, and white sturgeon were used in 10 trials and fish were injected 1 or 2 times with

LHRH_a; control fish were used in four trials. Results showed that there was 40 - 100% ovulation in treated fish; and no ovulation in the control fish. Overall, treatment appeared effective in nine trials and was inconclusive in one trial.

3. Dose: 100 ug/kg

Alligator gar, American shad, blue catfish, channel catfish, flathead catfish, lake sturgeon, paddlefish, and white sturgeon were used in 14 trials, fish were injected 1 or 2 times with LHRH_a; four of the trials involved control fish. Results showed that there was 0 - 100% ovulation in treated fish; and 0 - 100% ovulation in control fish. Overall, treatments appeared efficacious in 12 trials and ineffective in two trials.

Overall treatment resulted in 0 - 100% ovulation in females treated and 0 - 100% ovulation in control fish. Treatment appeared efficacious in 29 trials, ineffective in three trials, and inconclusive in one trial.

2. Observed Toxicity

No toxicity or adverse effects relating to LHRH_a treatments were reported in 48 trials conducted in CY09. The investigator noted in two studies involving paddlefish some fish increased swimming speed and were easily agitated after injection.

3. Observed Withdrawal Period

All withdrawal times were either met or exceeded.

Current Study Protocol for LHRHa INAD #8061

Please see the attached current study protocol for LHRHa INAD #8061. Please note no changes have occurred to this study protocol.

Facility Sign-up List

Please see “Table 5. Facilities and Names of Investigators” for facilities that signed-up to participate in the LHRHa INAD #8061 during CY09. Facilities not listed in Appendix III-a of the current LHRHa INAD #8061 study protocol have been highlighted.

The following facility had LHRHa on station during CY09 but never used the drug:

1. Bears Bluff NFH
2. Dexter NFH & TC

Correspondence sent to LHRHa Participants

Please see the attached correspondence that was sent to all LHRHa participants after the AADAP Office received their sign-up form for calendar year 2009.

Number of Treated Fish under Treatment Use Authorization

Total number of fish treated during CY09 was 2,314. The total number of treated fish to count against the treatment use authorization dated August 15, 2003 is 5,311.

Summary of Study Results

LHRH_a was used in 50 efficacy trials to induce gamete maturation in 19 different fish species (n = 2,314 treated fish; 317 untreated control fish) at dosages ranging from 5.0 - 100 ug/kg bw. LHRH_a was administered using either 1 - 2 injections. Water temperature during treatments ranged from 42.0 - 82.0°F. Overall, results showed that LHRHa treatment appeared efficacious in 88% of the trials, ineffective in 10% of the trials, and inconclusive in 2% of the trials. Data from the CY09 trials support the results of previous Annual Report submissions under INAD #8061 that indicate that LHRH_a treatment was efficacious in inducing gamete maturation in a variety of fish species. Although it is anticipated that the majority of future efficacy data collected under INAD #8061 will also be ancillary data, efforts will be made to improve the quality of data whenever possible.

References

- Coy, D.H., E.J. Coy, A.V. Schally, J. Vilchez-Martinez, Y. Hirotsu, and A. Arimura. 1974. Synthesis and biological properties of D-Ala⁶, des Gly¹⁰ LH-RH ethylamide, a peptide with greatly enhanced LH and FSH releasing activity. *Biochemical and Biophysical Research Communication*. 57(2): 335-340.
- Donaldson, E.M., and G.A. Hunter. 1983. Induced final maturation, ovulation, and spermiation in cultured fish. Pages 351-403 in W.S. Hoar, D.J. Randall, and E.M. Donaldson, editors. *Fish physiology*, volume 9. Part B. Academic Press, New York.
- Goetz, F.W. 1983. Hormonal control of oocyte maturation and ovulation in fishes. In: *Fish Physiology Vol IX, Part B*. Eds. W.S. Hoar, D.J. Randall and E.M. Donaldson. Academic Press, New York. pp. 117-169.
- Hoar, W.S. 1969. Reproduction. In: *Fish Physiology Volume III*. Eds. W.S. Hoar and D.J. Randall. Academic Press, New York and London. pp.1-72.

Table 1. Summary of Year 2009 LHRH_a Male Efficacy Results

Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval (hrs)	Observed Withdrawal Period (days)	Treated			Control	
							Number Treated	Dose (ug/Kg b.w.)	% Spermiat	Number of Controls	% Spermaite
1	effective	ALG	Private John Allen NFH	2	26	14	4	100	100	0	-
1	effective	AMS	Orangeburg NFH	1	1-5 days	ethanized	80	100	35 - 100 (ave. 69%)	60	33-100 (ave. 70)
1	effective	ASN	Manning SFH	1	24	No release	13	30	31	0	-
1	ineffective	FCF	Heart of the Hills Research	1	-	No release	10	100	0	10	0
1	effective	GIT	Mora NFH & TC	1-2	7 - 14 days	No release	20	5	95	20	85
1	effective	HNS	Bozeman FTC	1	12 - 18	ethanized	5	20	80	0	-
1	effective	HNS	Bozeman FTC	1	24	ethanized	10	50	100	0	-
1	ineffective	LMB	Meade SFH	1	1 - 14 days	No release	10	25	0	10	0
1	effective	LST	NYSDEC Region 6	1	20	15	9	10	100	0	-
1	effective	PAH	Aquaculture of Kentucky	1	20	No release	9	50	67	0	-
1	effective	PAH	Aquaculture Research Center	1	12 - 24	No release	6	50	100	0	-
1	effective	PAH	Booker Fowler SFH	1	12	ethanized	15	10	100	0	-
1	effective	PAH	Private John Allen NFH	2	15	Fish were released into a no take area	12	100	83	0	-
1	effective	PAH	Tishomingo NFH	1	30	45	23	25	80 - 100 (ave. 91%)	3	0
1	effective	PLS	Bozeman FTC	1	24	T&E species not for human consumption	4	20	100	0	-
1	effective	WST	Blind Canyon Aquaranch	1	30.5	No release	8	20	38	0	-
1	effective	WST	Sterling Caviar	1	20	1 yr	10	10	50	10	0

Table 2. Summary of Year 2009 LHRH _a Female Efficacy Results							Treated			Control	
Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval (hrs)	Observed Withdrawal Period (days)	Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	effective	ALG	Private John Allen NFH	2	26	14	3	100	67	0	-
1	effective	AMS	Orangeburg NFH	1	1-5 days	euthanized	95	100	100	65	100
1	ineffective	BCF	Jubilee Farms	2	-	14	32	100	0	0	-
1	effective	CCF	Catfish Genetics Research Unit	1 - 2	30-42	90	168	100	0 - 100 (ave. 45)	64	0
1	effective	CCF	Jubilee Farms	2	26	14	156	100	94 - 100	0	-
1	effective	CCF	NeedMore Fisheries	2	25	no release	844	100	35 - 78 (ave. 59)	0	-
1	ineffective	FCF	Heart of the Hills Research Station	1	-	no release	10	100	0	10	0
1	effective	GIT	Mora NFH & TC	1 - 2	7 - 14 days	no release	20	5	90	20	95
1	inconclusive	HNS	Bozeman FTC	2	24	euthanized	10	50	40	0	-
1	effective	HNS	Bozeman FTC	2	24	euthanized	19	20	89	0	-
1	ineffective	LMB	Meade SFH	1	1-14 days	no release	10	25	0	10	0
1	effective	LST	NYSDEC Region 6	2	20	15	2	50	100	0	-
1	effective	LST	Wild Rose SFH	2	24	120	8	100	67 - 100 (ave. 88)	2	50
1	effective	PAH	Aquaculture of Kentucky	2	12	no release	11	100	40 - 100 (ave. 73)	0	-
1	effective	PAH	Aquaculture Research Center	2	12 - 24	no release	4	100	100	0	-
1	effective	PAH	Booker Fowler SFH	2	12	euthanized	3	100	100	0	-
1	effective	PAH	Osage Catfisheries	2	24	no release	5	100	100	0	-

Table 2. Summary of Year 2009 LHRH_a Female Efficacy Results

							Treated			Control	
Number of Trials	Efficacy	Species	Facility	Number of Injections	Spawning Interval (hrs)	Observed Withdrawal Period (days)	Number Treated	Dose (ug/Kg b.w.)	% Ovulate	Number of Controls	% Ovulate
1	effective	PAH	Private John Allen NFH	2	15	Fish were released into a no take area	4	100	100	0	-
1	effective	PAH	Tishomingo NFH	1	30	45	9	25	78	3	0
1	effective	PAH	Tishomingo NFH	1	30	45	18	50	61	0	-
1	effective	PLS	Bozeman FTC	2	24	T&E species not for human consumption	2	50	100	0	-
1	effective	RBT	Fall River SFH	1	32	100	535	17	82	0	-
1	effective	SAR	Charlie Craig SFH	2	7 days	euthanized	11	50	46	9	0
1	effective	SBS	Evan Fish Farm	2	36	>14	3	40	100	3	0
1	effective	STB	Blackwater Fisheries Research	1	3	no release	8	25	0 - 100 (ave. 63)	0	-
2	effective	STB	Milford SFH	1	25 - 53	90	33	20	67 - 86	0	-
1	effective	STB	Milford SFH	1	29 - 54	90	11	34	82	0	-
1	effective	SVN	Evan Fish Farm	2	30	euthanized	3	40	50 - 100	3	0
1	effective	WAE	Charlie Craig SFH	1	32 - 60	euthanized	10	50	100	9	0
1	effective	WST	Blind Canyon Aquaranch	2	30	no release	4	50	50	0	-
1	effective	WST	Kootenai Tribal Hatchery	2	27 - 34	T&E species not for human consumption	9	100	100	0	-
1	effective	WST	Sterling Caviar	2	20	1 yr	6	20	67	6	0

Table 3. Description of Number of Treatment Trials, the Number of Fish and Species Treated, and Treatment Regimens used During CY 2009 LHRH_a Efficacy Studies

Total Number of Treatment Trials	50
Number of Trials that Appeared Efficacious:	44 (88%)
Number of Trials that Appeared Inefficacious:	5 (10%)
Number of Trials that were Inconclusive	1 (2%)

Total Number of Treated Fish:	2,314
Number of fish treated in efficacious trials	2,232
Number of fish treated in ineffective trials	72
Number of fish treated in inconclusive trials	10

Treatment Regimes Used:	
5 - 25 ug/Kg body weight (1 - 2 injections)	18 trials
50 ug/Kg body weight (1 - 2 injections)	14 trials
100 ug/Kg body weight (1 - 2 injections)	18 trials

Water Temperature (°F) Range: 42.0 - 82.0

Fish Species Treated:

Salmonids

Gila trout *Oncorhynchus gilae* rainbow trout *O. Mykiss*

Non-salmonids

alligator gar <i>Lepisosteus spatula</i>	American shad <i>Alosa sapidissima</i>
Atlantic sturgeon <i>Acipenser oxyrhynchus</i>	blue catfish <i>Ictalurus furcatus</i>
lake sturgeon <i>A. fulvescens</i>	channel catfish <i>I. punctatus</i>
Siberian sturgeon <i>A. baerii</i>	flathead catfish <i>Pylodictis olivaris</i>
starry sturgeon <i>A. stellatus</i>	largemouth bass <i>Micropterus salmoides</i>
white sturgeon <i>A. transmontanus</i>	striped bass <i>Morone saxatilis</i>
pallid sturgeon <i>Scaphirhynchus albus</i>	paddlefish <i>Polydon spathula</i>
shovelnose sturgeon <i>S. platyrhynchus</i>	sauger <i>Stizostedion canadense</i>
walleye <i>Sander vitreus</i>	

Size Class of Treated Fish: Adults